IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.:	**DEX-0201**
Inventors:	**Yang et al.**
Serial No.:	**09/817,607**
Filing Date:	**26 March 2001**
Examiner:	**Harris, Alana M.**
Group Art Unit:	**1642**
Title:	**Compositions and Methods for Diagnosing, Monitoring, Staging, Imaging and Treating Colon Cancer**

Declaration by Dr. Roberto Macina

I, Roberto Macina, hereby declare:

1. I was awarded a M.S in Biology, and a Ph.D. in 1990 in Molecular Biology from the University of Buenos Aires, Argentina. After obtaining these degrees, I spent four years at The Wistar Institute, University of Pennsylvania contributing to the Human Genome Project endeavor. From 1995 to 1997, I served in the Molecular Diagnostic Department at SmithKline Beecham holding the positions of Investigator and Senior Investigator. Since the inception of diaDexus, Inc. in 1997 I have served as the Assistant Director of Cancer Gene Discovery. In October 2001 I assumed the position of Director of Molecular Technologies at diaDexus, Inc.

2. As the Director of Molecular Technologies for diaDexus, Inc., and a named inventor, I am familiar with the teachings of the above-referenced patent application.

3. Since filing of the above-referenced patent application, the Gene Discovery division at diaDexus Inc. has performed experiments confirming the utility of SEQ ID NO:8 (sqcln017, Cln136) with regard to colon cancer.

4. SEQ ID NO:8 (sqcln017, Cln136) relative expression analysis was performed in accordance with a standard Quantitative Polymerase Chain Reaction (QPCR) protocol well known to those of skill in the art prior to March 2000, and outlined in the above-referenced patent application at page 22, lines 16-24; page 40, lines 1-14; and page 41, lines 14-31.

5. I personally supervised experiments to measure the relative levels of sqcln017 (Cln136) in cancerous, normal-adjacent, and normal tissues. In these experiments, relative quantitation of gene expression was done using Polymerase Chain Reaction in real time.

Quantitative PCR with fluorescent Taqman® probes is a quantitation detection system utilizing the 5′- 3′ nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman®) labeled with a 5′ reporter dye and a downstream, 3′ quencher dye. During PCR, the 5′-3′ nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA). Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ATPase, or 18S ribosomal RNA (rRNA) is used as this

endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample were used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" can be obtained using the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

The tissue distribution and the level of mRNA expression of the target gene are evaluated for every sample in normal and cancer tissues. Total RNA is extracted from normal tissues, cancer tissues, and from cancers and the corresponding matched adjacent tissues. Subsequently, first strand cDNA is prepared with reverse transcriptase and the polymerase chain reaction is done using primers and Taqman® probes specific to each target gene. The results are analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the target gene in a particular tissue compared to the calibrator tissue.

One of ordinary skill can design appropriate primers. The relative levels of expression of SEQ ID NO:8 versus normal tissues and other cancer tissues can then be determined. All the values are compared to the calibrator. Normal RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

The relative levels of expression of the SEQ ID NO:8 in pairs of matched samples may also be determined. A matched pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual. All the values are compared to the calibrator.

6. Results from this QPCR are depicted in the attached expression graphs.

The expression graph shows that the levels of sqcln017 (Cln136) are higher in the cancer samples tested when compared with all the normal tissues and the normal adjacent tissues for colon cancer. The sensitivity calculated comparing the levels of Cln136 in the colon cancer samples versus the expression in the colon normal adjacent tissue from the same patient is 41% (41% of the cancer samples show levels of sqcln017 (Cln136) at least 2 fold higher than the corresponding normal adjacent form the same patient). The sensitivity calculated comparing the cancer samples versus the normal colon sample is of 56%. The specificity for sqcln017 (Cln136) is of 43%. This specificity is an indication of the level of colon tissue specific expression of the gene compared to all the other tissue types tested in our assay. Thus, these additional experiments confirm the usefulness SEQ ID NO:8 (sqcln017, Cln136) with regard to colon cancer.

7. SEQ ID NO:8 (sqcln017, Cln136) is at least as specific and sensitive as many useful cancer therapeutics and diagnostics that have been FDA approved and are commercially available. For example, Genentech's product Herceptin® and its diagnostic counterpart, the HercepTest® are very successful commercially. Yet many publications show the relevant gene, HER-2, is overexpressed in 30% of breast cancer patients. Hence, in my professional opinion the specificity and sensitivity of SEQ ID NO:8 (sqcln017, Cln136) is sufficient for use with regard to colon cancer.

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information or belief are believed to be true; and further

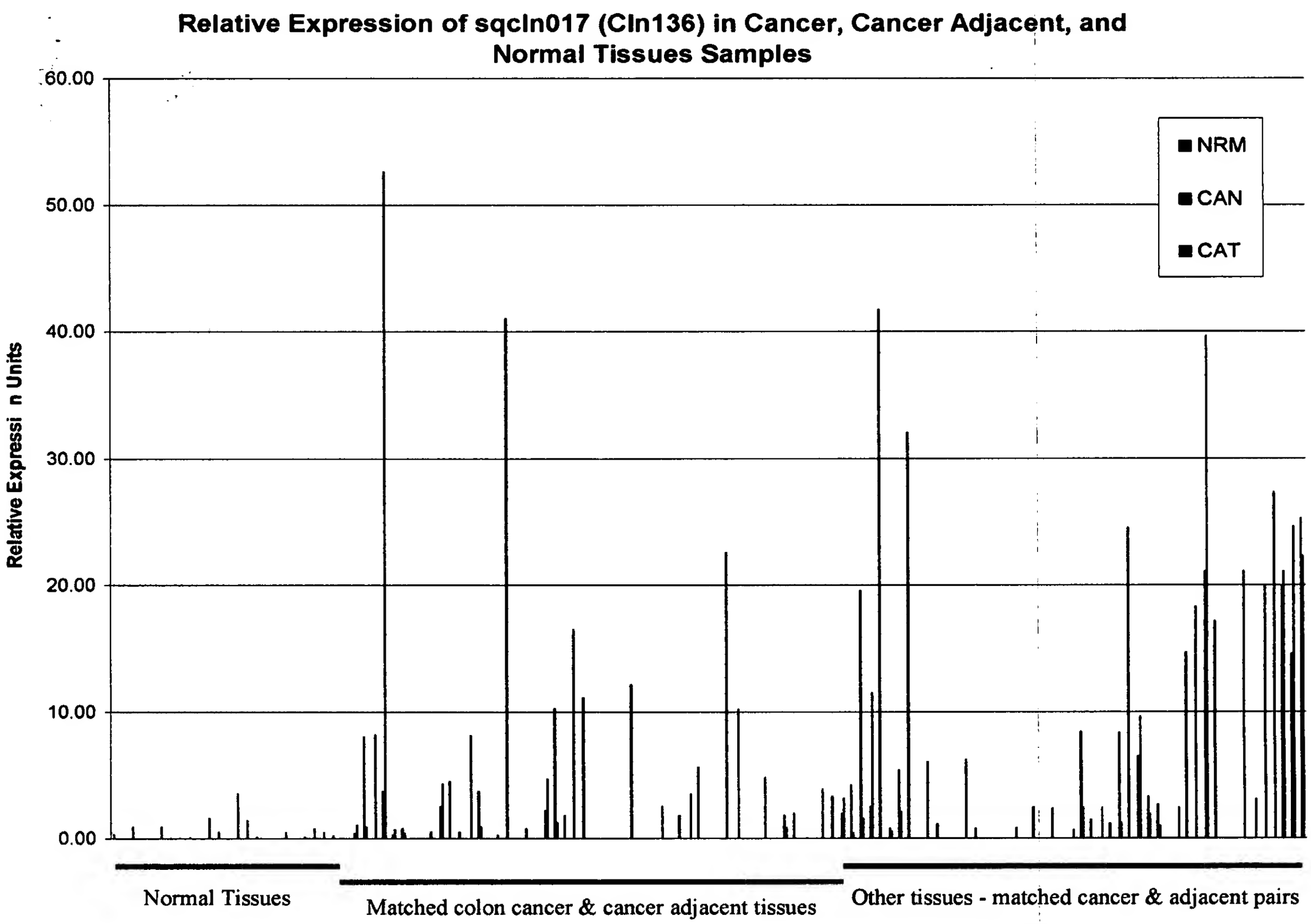
that these statements were made with the knowledge that willful statements and the like so made are punishable by fine or by imprisonment, or both, under §1001 of Title 18 of the United States code, and that such willful statements may jeopardize the validity of the application, any patent issuing there upon, or any patent to which this verified statement is directed.

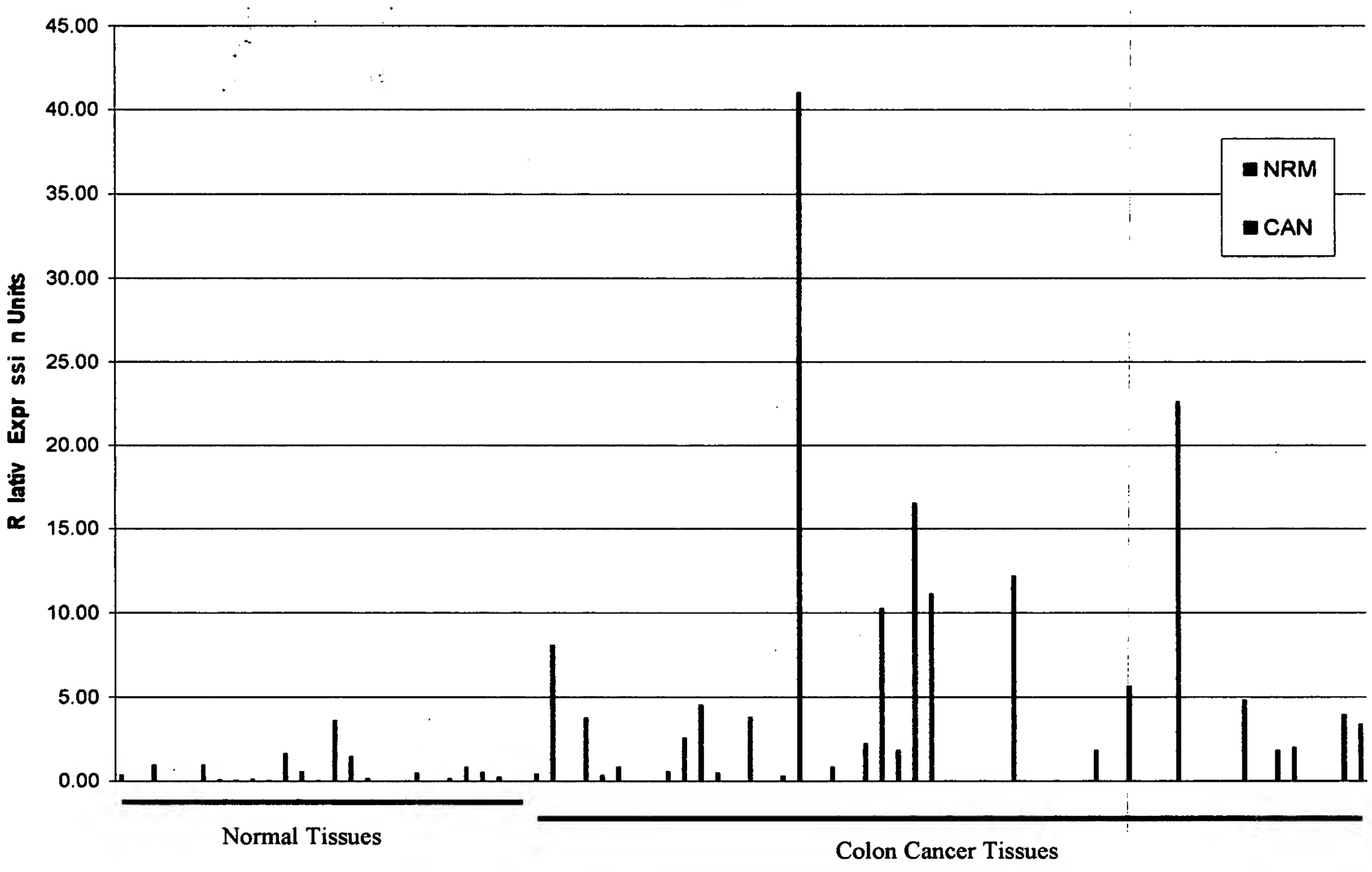
_____

Roberto Macina, Ph.D.

_____

Date


Relative Expression of sqcIn017 (CIn136) in Cancer, Cancer Adjacent, and Normal Tissues Samples
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Relative Expressi n Units
NRM
CAN
CAT
Normal Tissues
Matched colon cancer & cancer adjacent tissues
Other tissues - matched cancer & adjacent pairs


Relative Expression of sqcIn017 (CIn136) in Colon Cancer vs Normal Tissue Samples
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
R lativ Expr ssi n Un ts
NRM
CAN
Normal Tissues
Colon Cancer Tissues